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Molecular Docking Studies of Flavonoid Bioactive Compound against RNA Polymerase *Mycobacterium tuberculosis.*

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ABSTRACT

Tuberculosis (TB) remains one of the world's deadliest communicable diseases. In 2013, an estimated 9 million people developed TB and 1.5 million died from the disease. Rifampicin resistance is caused because of the change of the structure beta subunit of DNA dependant RNA polymerase. In this paper we studied the bioactive compounds of flavonoid-RNA Polymerase receptor interactions *In silico*. First, homology modeling was performed to obtain the three dimensional structure of RNA Polymerase *Mycobacterium tuberculosis*. Preparation of sixth bioactive compounds of flavonoid which will be as ligands, Rifapentin as a comparison. The sixth bioactive compounds of flavonoid, and Rifapentine were docked with beta subunit of RNA polymerase *Mycobacterium tuberculosis* until energy values were obtained. The sixth bioactive compounds of flavonoid had lesser energy values than rifapentin, sixth bioactive flavonoid compounds also predicted to have greater binding affinity to RNA polymerase *Mycobacterium tuberculosis.*

Keywords: Tuberculosis, RNA polymerase, Flavonoids, Antituberculosis, Docking

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INTRODUCTION

Tuberculosis (TB) remains a major global health problem, reponsible for ill health among millions of people each year. TB ranks as the second leading cause of death from an infectious disease worldwide. The latest estimates included in this report are that there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths. The six countries that stand out as having the largest number of incident cases in 2013 were India (2.0 million–2.3 million), China (0.9 million–1.1 million), Nigeria (340.000−880.000), Pakistan (370.000−650.000), Indonesia (410.000−520.000) and South Africa (410.000−520.000) [1].

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. Isoniazid (INH), rifampicin (or other rifamycin), pyrazinamide, ethambutol, and streptomycin are the five first-line agents for treatment of tuberculosis. An Isoniazid-Rifampicin combination administered for 9 months will cure 95–98% of cases of tuberculosis caused by susceptible strains, Rifampicin is a semisynthetic derivative of rifamycin, Rifampicin binds to the β subunit of bacterial DNA-dependent RNA polymerase and thereby inhibits RNA synthesis [2]. *Mycobacterium tuberculosis* are intrinsically resistant to most antibiotics. Because they grow more slowly than other bacteria, antibiotics that are most active against rapidly growing cells are relatively ineffective. *Mycobacterium* cells can also be dormant and thus completely resistant to many drugs or killed only very slowly. The lipid-rich mycobacterial cell wall is impermeable to antibiotic and other agents. Mycobacterial species are intracellular pathogens, and organisms residing within macrophages are inaccessible to drugs that penetrate these cells poorly. Finally, mycobacteria are notorious for their ability to develop resistance. Multidrug resistant tuberculosis (MDR-TB) had been wide reported. Worldwide, the proportion of new cases with multidrug-resistant TB (MDR-TB) was 3.5% in 2013. Treatment for multidrug-resistant TB (MDR-TB), defined as resistance to isoniazid and rifampicin (the two most powerful anti-TB drugs) is longer, and requires more expensive and more toxic drugs. Resistant of Rifampicin results from any one of several possible point mutations in *rpoB*, the gene for the β subunit of RNA polymerase [2]. Many research groups have gone isolated and identified the structure from natural product to discover new antituberculosis drug to stop epidemic tuberculosis in the World [2].

Flavonoids are natural bioactive compounds found in fruit, vegetables, nuts, seeds, stems and flowers as well as tea, wine, propolis and honey. Flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity [3], antifungal activity, antivirus activity [4], antiallergic activity, antioxidant activity [5] [6], vascular activity [7] and cytotoxic antitumour activity. Many research groups have gone one step further and either isolated and identified the structure of flavonoids that possess antibacterial activity. Examples of such flavonoids are (+)-catechin, rutin [8], quercetin [4], quercitrin, kaempferol, and luteolin [9].

In this paper we studied the bioactive compounds of flavonoid-RNA Polymerase receptor interactions In silico. This in silico method commonly used in the initial screening bioactive compounds for drug candidates [10]. Moleculer docking as an initial screening process between the molecules of bioactive compounds that bind to the active site of RNA polymerase *Mycobacterium tuberculosis*. In silico methods are widely used in early research to discovery of anticancer [11], antivirus [10] [12] and discovery of bioactive compounds that can be used as a drug candidate [13] [14].

MATERIALS AND METHODS

Preparation The bioactive compounds of Flavonoids

(+) Catechin, rutin, quercetin, quercitrin, kaempferol, and luteolin is bioactive compounds of flavonoid used as ligand. The sixth structure of active compounds is downloaded from PubChem database (http://pubchem.ncbi.nlm.nih.gov) in file format pdb. The sixth flavonoid compounds were prepared by removal H2O cluster, addition of hydrogen cluster using discovery studio 4.1 software and structure sixth bioactive compounds of flavonoid is stored in a file format pdb and mol2.

Preparation of Rifapentine as comparison ligands

Rifapentine was antibiotic for tuberculosis as comparison ligand, structure of rifapentin downloaded from PubChem database (http://pubchem.ncbi.nlm.nih.gov/). This structure were prepared by removal H2O

cluster, addition of hydrogen cluster using discovery studio 4.1 software and stored in a file format pdb and mol2.

Search for Beta subunit RNA polymerase (rpoB) of similar sequences and homology modeling RNA polymerase Mycobacterium tuberculosis (3D structure prediction of RNA Polymerase)

The search for sequences similar to RNA Polymerase subunit Beta *Mycobacterium tuberculosis* within the Protein Data Bank (PDB) was performed with the Basic Local Alignment Search Tool program (BLAST : http://blast.ncbi.nlm.nih.gov/Blast.cgi). The search for the best template for modeling was carried out by choosing structures possessing a high degree of sequence similarity with RNA Polymerase Mycobacterium tuberculosis. The crystal structural coordinates of RNA polymerase of *Thermus thermophilus* (*tt*RNAP) at 2.5 Å resolution (PDB code: 2A69) was used as template structure to build a three-dimensional model of RNA Polymerase *Mycobacterium tuberculosis* [15]. Sequence alignment was performed using clustalo (http://www.ebi.ac.uk/Tools/msa/clustalo/) with manually adjusted. 3D structure of RNA polymerase of *Mycobacterium tuberculosis* designed using the Swiss-model server (http://swissmodel.expasy.org/). Modeling results using Swiss-model server downloaded and stored in file format pdb.

Determination of Active Sites for ligand binding site

Site Active site residues were confirmed by relying on a study of Josa *et* al [15].

Validation of 3D Structure of RNA Polymerase

Validation of 3D structure of RNA Polymerase obtained from homology modeling by PROCHECK server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) for Ramachandran plot analysis[21].

Moleculer Docking between ligand (sixth bioactive compounds of flavonoid) and RNA Polymerase Mycobacterium tuberculosis

The docking studies were carried out with autodock vina ver. 1.1.2 and Autodock ver. 4.2.6 software, a free program for predicting the most likely conformation of how a ligand will bind to a macromolecule [16] [17]. Default parameters were used for the docking process and Binding energy values of each docking were obtained.

Visualization of the Moleculer Docking result

Visualization of the results with Yasara and Pymol software.

RESULT AND DISCUSSION

Preparation The bioactive compounds of Flavonoids

The sixth structure of active compounds is downloaded from PubChem database (http://pubchem.ncbi.nlm.nih.gov) in file format pdb. (+)-Catechin, rutin, quercetin, quercitrin, kaempferol, and luteolin were prepared by removal H2O cluster, addition of hydrogen cluster using discovery studio 4.1 software. This structure of sixth bioactive compounds of flavonoid shown as follows :

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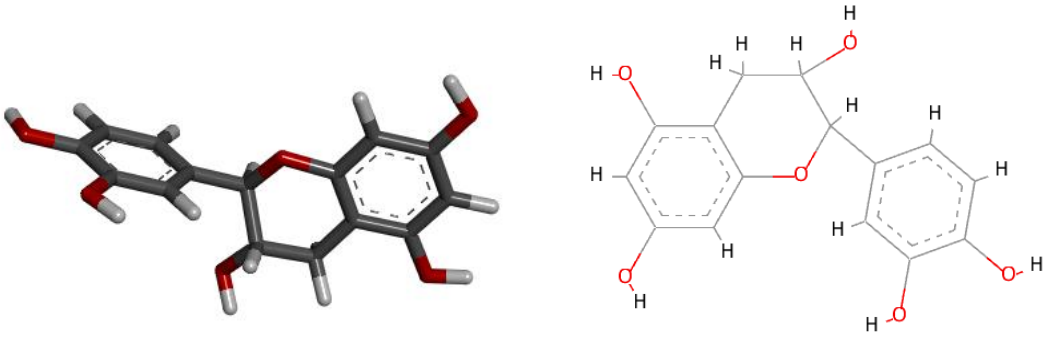

Fig 1. (+)-Catechin (Pubchem CID : 9064)

(a) 3D Structure (b) 2D Structure Molecular Formula : C15H14O6 MW : 290.26806 g/mol

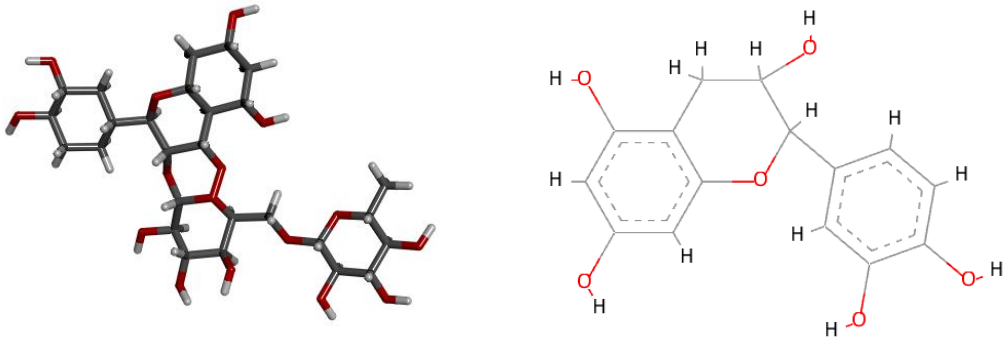

Fig 2. Rutin (Pubchem CID : 5280805)

(a) 3D Structure (b) 2D Structure Molecular Formula : C27H30O16 MW : 610.5175 g/mol

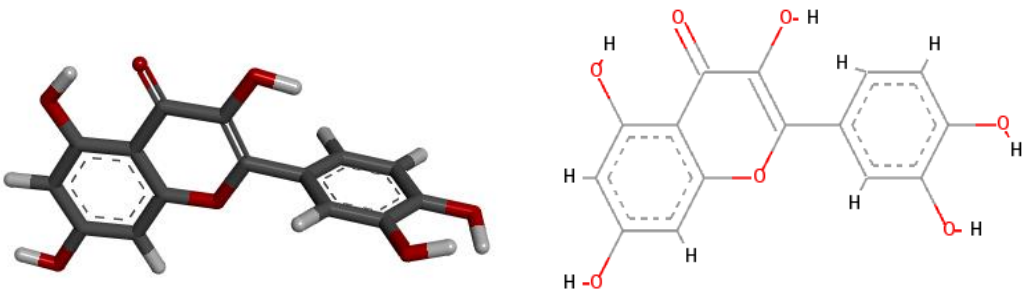

Fig 3. Quercetin (Pubchem CID : 5280343)

(a) 3D Structure (b) 2D Structure Molecular Formula : C15H10O7 MW : 302.2357 g/mol

ISSN: 0975-8585

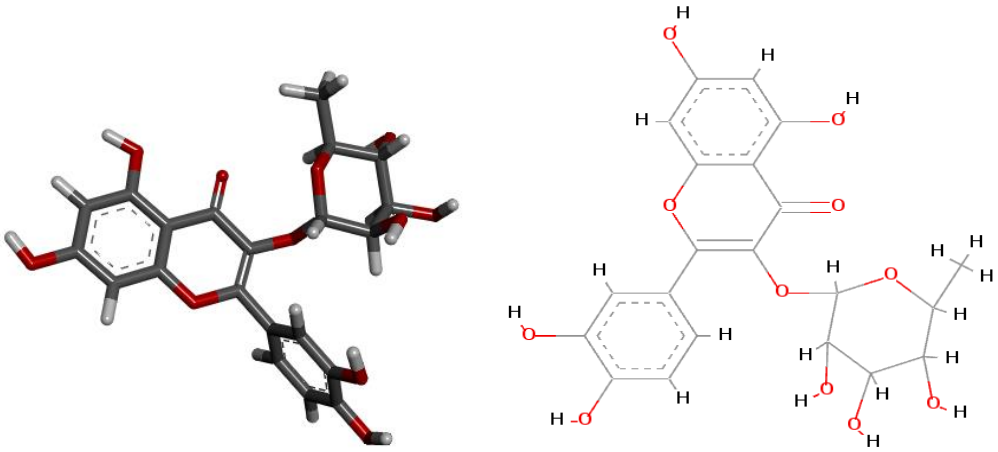

Fig 4. Quercitrin (Pubchem CID : 5280459)

(a) 3D Structure (b) 2D Structure Molecular Formula : C21H20O11 MW : 448.3769 g/mol

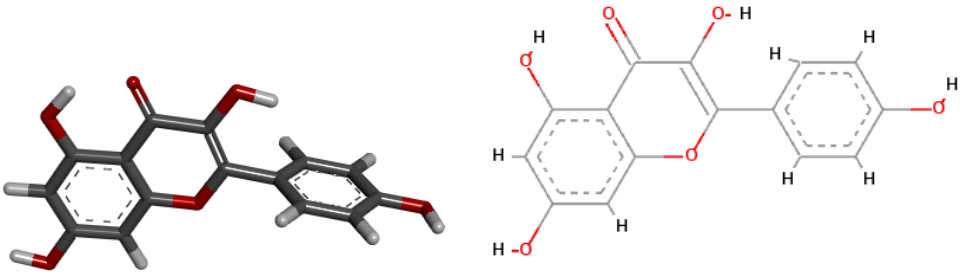

Fig 5. Kaempferol (Pubchem CID : 5280863)

(a) 3D Structure (b) 2D Structure Molecular Formula : C15H10O6 MW : 286.2363 g/mol

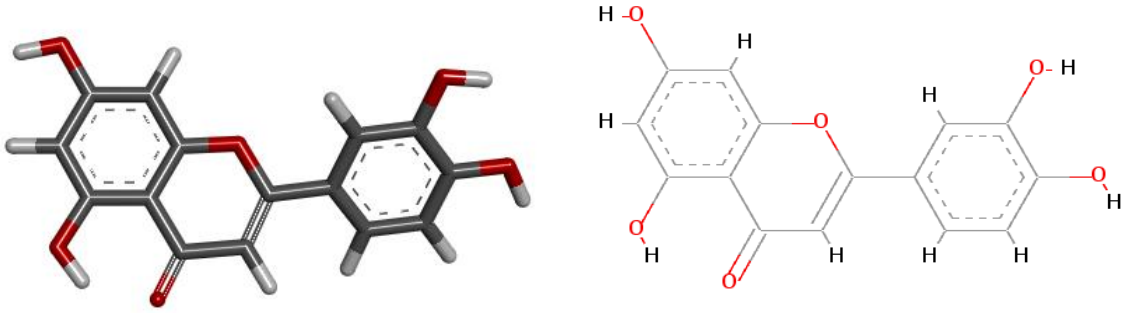

Fig 6. Kaempferol (Pubchem CID : 5280445)

(a) 3D Structure (b) 2D Structure Molecular Formula : C15H10O6 MW : 286.2363 g/mol

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Preparation of Rifapentine as comparison ligand

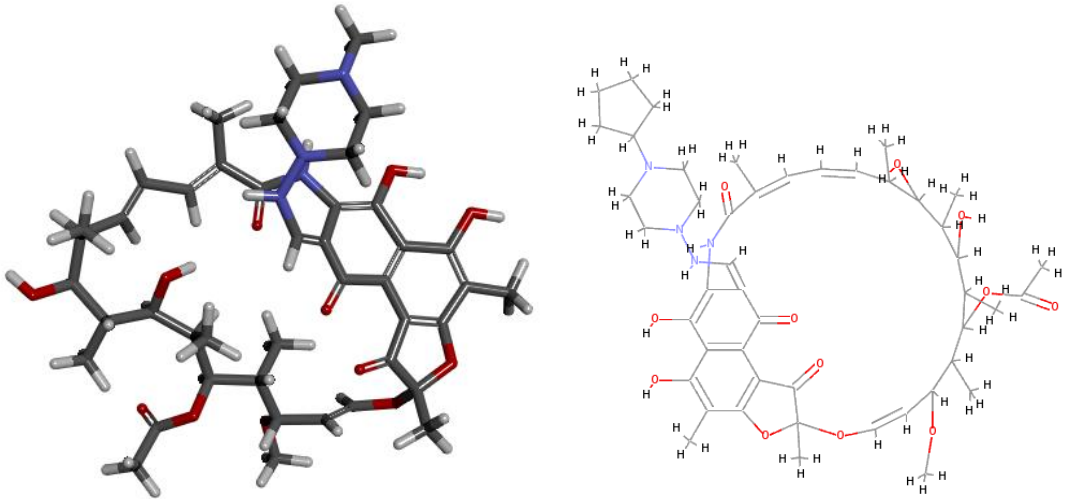

Fig 8. Rifapentine (Pubchem CID : 6323497)

(a) 3D Structure (b) 2D Structure Molecular Formula : C47H64N4O12 MW : 877.03066 g/mol

Rifapentine is a semisynthetic derivative of rifamycin, both are antibiotics used as anti-tuberculosis drugs [2]. The following is the structure of rifapentine :

Search for Beta subunit RNA polymerase (rpoB) of similar sequences and homology modeling RNA polymerase Mycobacterium tuberculosis (3D structure prediction of RNA Polymerase)

Twenty two BLAST (RID : 1SFW77X0014) hits were obtained for the query rpoB sequence those including, *Thermus aquaticus* RNA Polymerase holoenzyme (1L9U), Taq RNA Polymerase-Sorangicin Complex (1YNJ), crystal structure of *Thermus aquaticus* core RNA Polymerase (1HQM), *Thermus aquaticus* Core RNA Polymerase Rifampicin Complex (1I6V), Crystal Structure Of The RNA Polymerase Holoenzyme From *Thermus thermophilus* At 2.6a Resolution (1IW7) that have a high level of sequence identity. All the a for ementioned protein sequences showed 65% sequence identity to our query RpoB sequence. Multiple sequence alignment performed by submitting *Mycobacterium* rpoB (RNAP_C) and the homologues obtained from BLAST search as input files to Clustal omega server inferred that *Thermus thermophilus* RNA Polymerase chain C (2A69_C) [19] and *Mycobacterium* rpoB are closest homologues. JalView application of clustal omega was used to view the phenetic tree created based on neighbor joining algorithm [20]. The phylogram tree (Fig.9) showed that *Thermus thermophilus* RNA Polymerase (2A69_C) and *Mycobacterium* rpoB have close evolutionary relationship of all homologues.

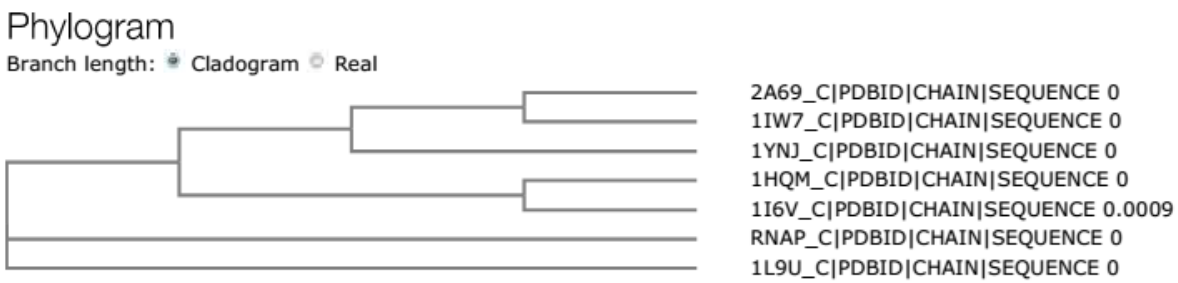

Fig 9. Phylogram RNAP

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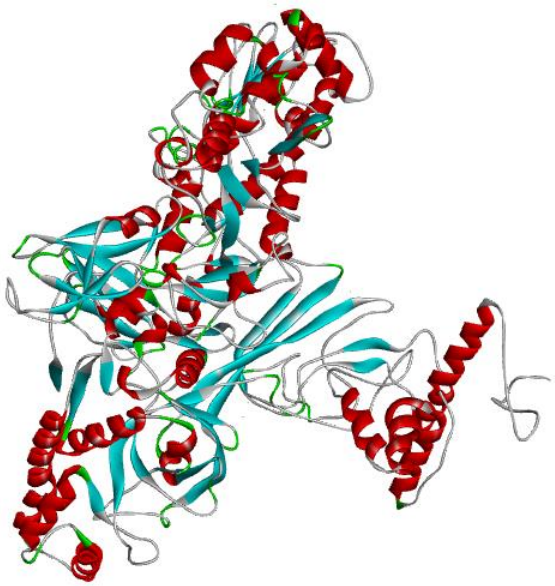

Fig 10. 3D Structure of RNA polymerase

As the 3D structure for *Mycobacterium* RNA Polymerase protein is not available in structural databases, its 3D structure was generated using the Swiss-model server. Follows the structure of RNA polymerase homology modeling results with swiss-model server :

Validation of 3D Structure of RNA Polymerase

The Ramachandran plot of the RNA Polymerase *Mycobacterium tuberculosis* (Figure 11) satisfied the tests with 94.0% (1050 residues) of the residues in the most favored regions, 4.7% (53 residues) in additional allowed regions and 1.3 % (14 residues) of the residues in outliner region. The RNA Polymerase *Mycobacterium tuberculosis* quality is good and can be used for docking.

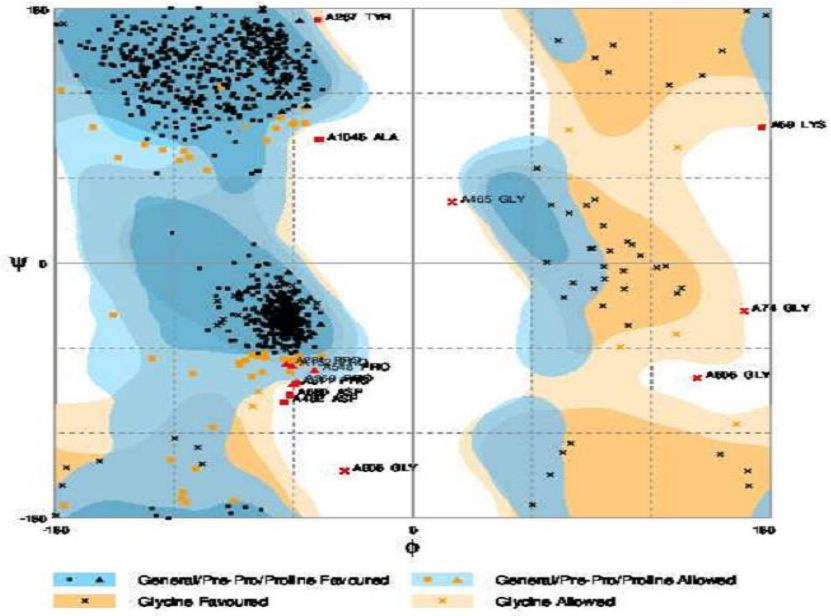

Fig 11. *Ramachandran Plot* from RNA *Polymerase Mycobacterium tuberculosis* generate with Procheck server

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Ramachandran plot is used to check and adjust a model of protein conformation. Ramachandran plot has an area marked with blue lines around it, which is the secondary structure of proteins coordinate area as an area of maximum tolerance limits steric strain. This area as an area that is allowed (allowed regions), where the protein is beyond the blue line, then the amino acids are in the outliner (disallowed region). In the area of the plot tolerance limit residue other than glycine is not allowed, because glycine has no side chain, so that the angle ϕ (phi) and the angle ψ (psi) generated infinite. The number of plots residue other than glycine if the prohibited area indicates the quality of the protein structure. If the amount exceeds 15% of the entire amount of residual protein, the protein quality is not good and can not be used for further processing [18].

Moleculer Docking between ligand (sixth bioactive compounds of flavonoid) and RNA Polymerase Mycobacterium tuberculosis

Table I lists the binding energy values calculated between each compound and the target molecule obtained from the docking study. In RMSD value ≥ 2 angstroms sixth compounds has a lower binding energy (more stable complex) and it is a more potent inhibitor.

Table I: Moleculer Docking result with Autodock and autodock vina software

Ligand	Target Protein	*Binding Energy (Kcal/mol)* Autodock Vina 1.1.2	*Binding Energy (Kcal/mol)* Autodock 4.2.6
(+)-Catechin	RNA Polymerase	-6.7	-5.72
Quercetine	RNA Polymerase	-6.8	-4.94
Quercitrin	RNA Polymerase	-7.1	-5.18
Luteolin	RNA Polymerase	-6.9	-5.38
Kaempferol	RNA Polymerase	-6.5	-5.4
Rutin	RNA Polymerase	-6.8	-4.96
Rifapentine	RNA Polymerase	-6.0	-4.43

Binding Energy is also called Binding Affinity is the energy required by a ligand binds to the target protein. The smaller value of Binding Energy / Binding Affinity is the more stable a ligand binding to the target protein. RMSD value is a relative calculation of the best form of heavy atoms (atomic weight) that can move. RMSD calculation in Autodock vina there are two RMSD lower bound (RMSD the lowest bond) and an upper bound RMSD (RMSD the top bond). RMSD value of a heavy atom good if its value is ≥ 2 angstroms [16].

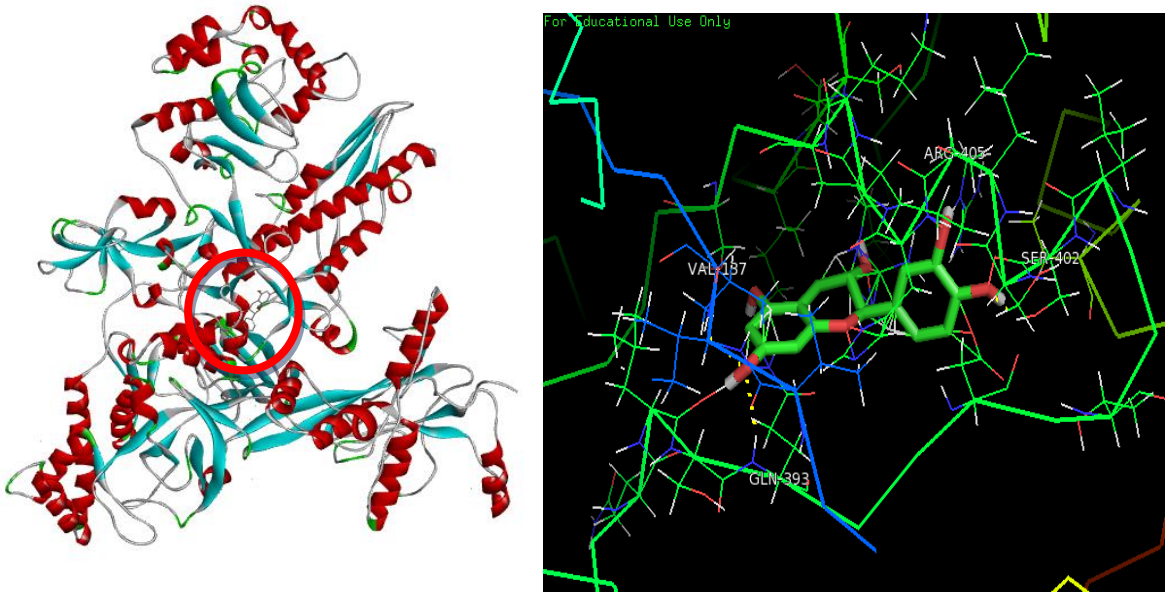

Fig.12. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond (+)-Cathecin with RNA *Polymerase* with *software pymol*

ISSN: 0975-8585

Visualization of the Moleculer Docking result

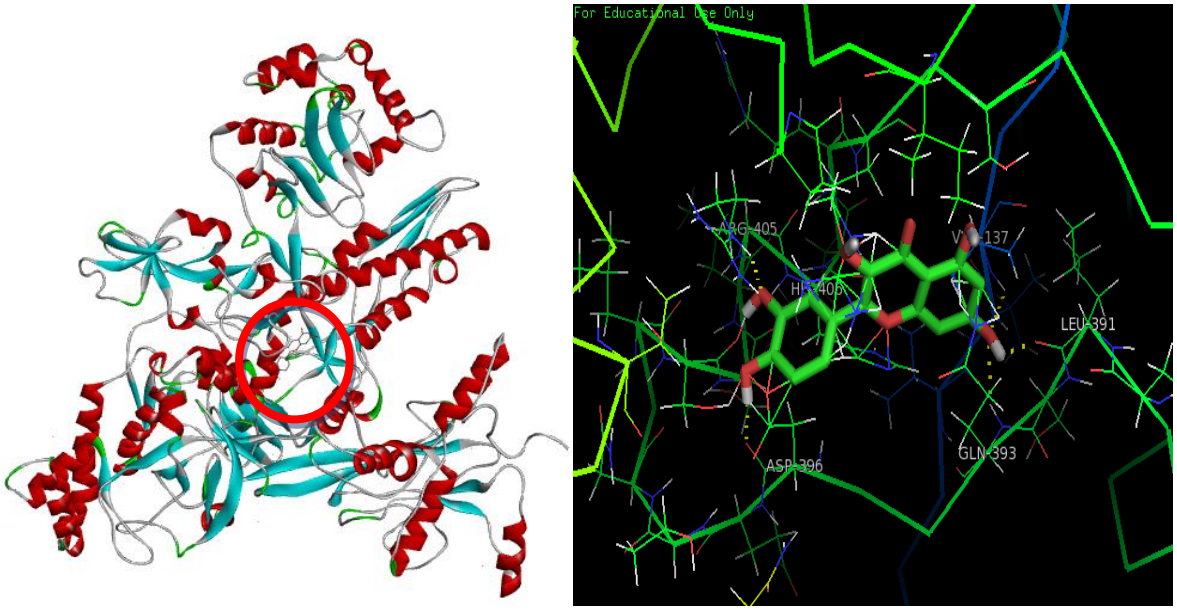

Fig.13. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond Quercetine with RNA *Polymerase* with *software pymol*

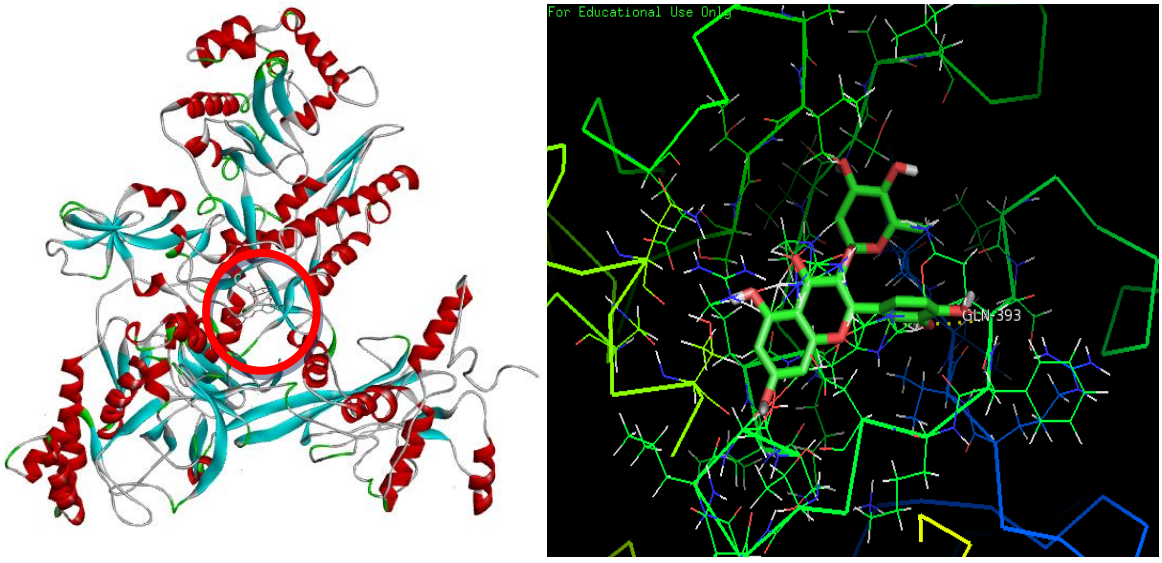

Fig.14. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond Quercitrin with RNA *Polymerase* with *software pymol*.

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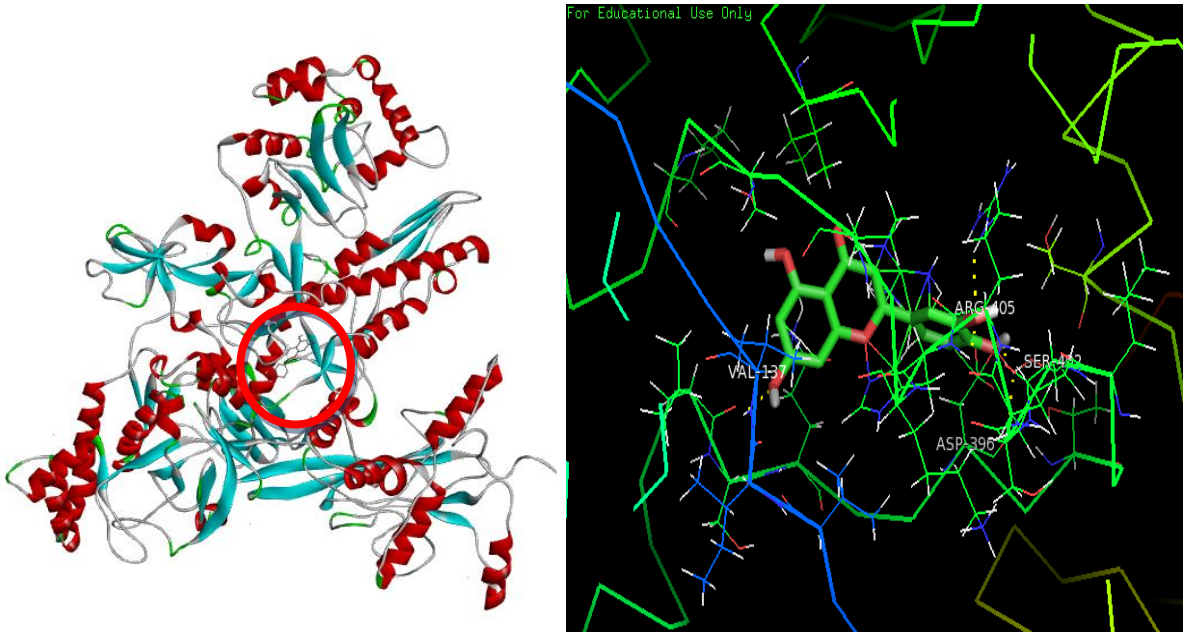

Fig.16. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond Luteolin with RNA *Polymerase* with *software pymol*

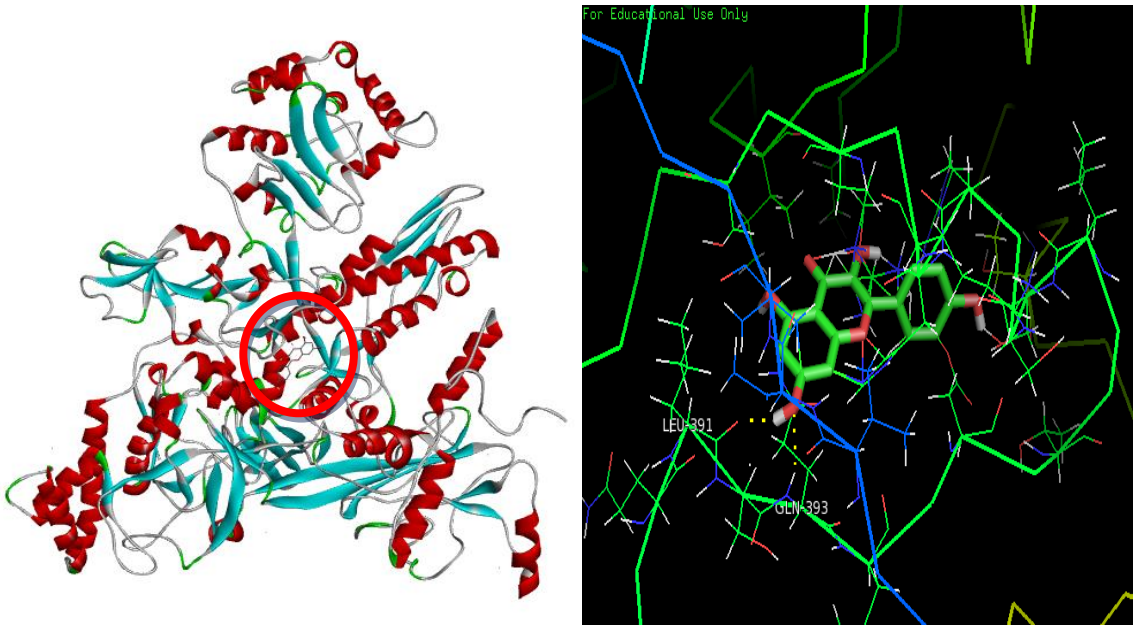

Fig.17. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond Kaempferol with RNA *Polymerase* with *software pymol*

ISSN: 0975-8585

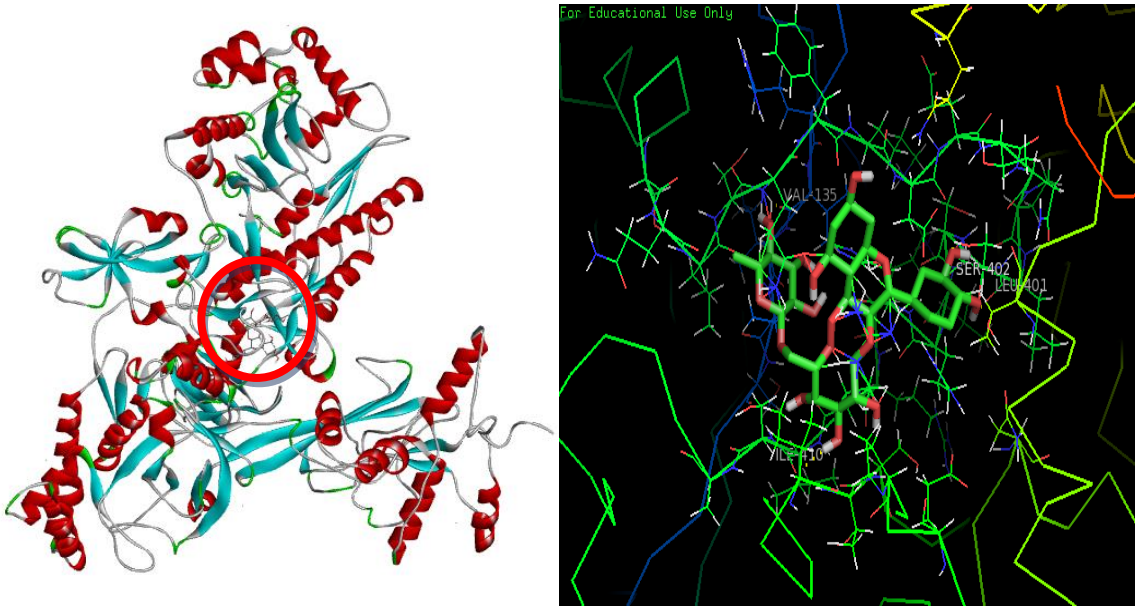

Fig.18. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond Rutin with RNA *Polymerase* with *software pymol*

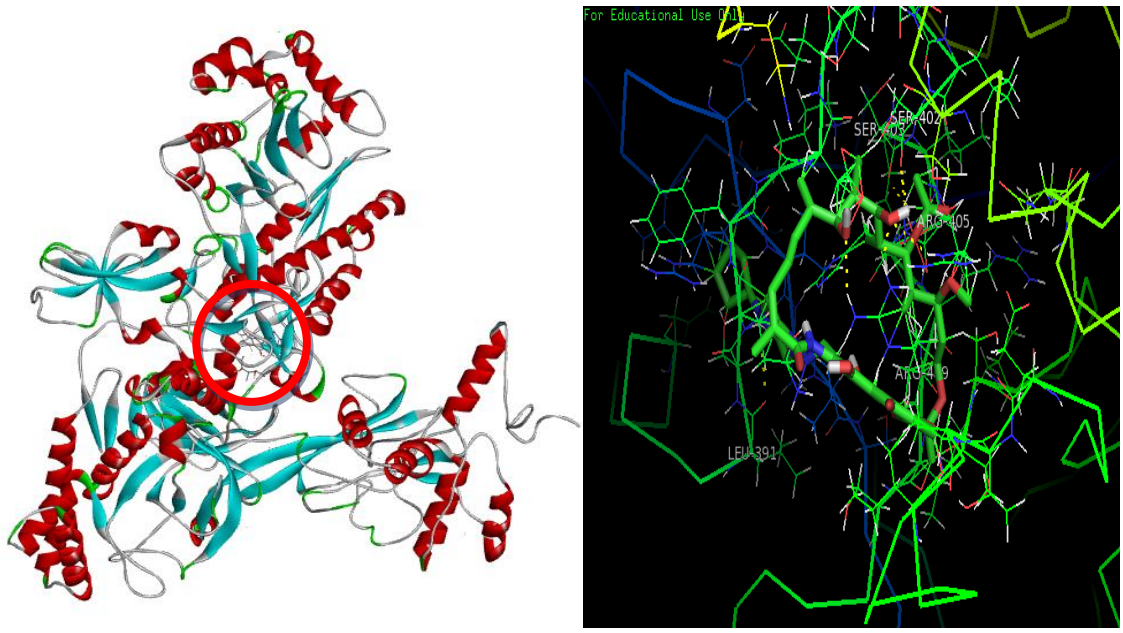

Fig.19. **(a)** Docking molecular result with *discovery studio* 4.1. *software* **(b)** molecular bond Rifapentine-RNA *Polymerase* with *software discovery studio* 4.1. **(c)** molecular bond Rifapentine with RNA *Polymerase* with *software pymol*

CONCLUSIONS

From this docking study we obtained sixth active coumpounds of flavonoid ((+) Catechin, rutin, quercetin, quercitrin, kaempferol, and luteolin) with binding energy values lesser than Rifapentine which means that these sixth active coumpounds of flavonoid are more compatible with RNA Polymerase receptor and may have better binding to the rpoB binding site compared to conventional Rifapentine.

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